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Antimicrobial Efficacy of *Jatropha Curcas Linn* and *Nicotiana Tabacum Linn* against Microorganisms from Wounds of Diabetic Patients

Asha O.A
Ph.D. Student, Department of Microbiology,
Federal University of Technology, Akure, Nigeria
A.K. Onifade
Professor, Department of Microbiology,
Federal University of Technology, Akure Nigeria
K. Oladunmoye
Professor, Department of Microbiology,
Federal University of Technology, Akure Nigeria

Abstract:
This study was designed to determine the antimicrobial efficacy of Jatropha curcas Linnand Nicotiana tabacum Linn against microorganisms from wounds of diabetic patients in Ondo State, Nigeria. A total of 454 wound swab samples collected from diabetic patients admitted at some government hospitals, the types and loads of microorganisms isolated from the samples were determined using growth-dependent and molecular methods. The antimicrobial sensitivity profile of isolates from wound swabs was evaluated against standard antibiotics using disc diffusion method. Thereafter, the antimicrobial efficacy of the plant extracts against isolates that showed multiple antibiotic resistance was determined using agar well diffusion technique. The molecular characteristics of some antibiotic resistant bacteria isolated were identified by extracting the Deoxyribonucleic acid (DNA) through the CTAB (Cetyltrimethyl Ammonium Bromide) method. DNA quality was checked using gel electrophoresis after which the DNA was amplified using PCR (Polymerase Chain Reaction) and the purified DNA was sequenced. Extracts were prepared from the different parts' of N. tabacum Linni and J. curcas Linniusing cold water, hot water, ethanol and n-Hexane as the extraction solvents, at concentrations of 25, 50, 75 and 100% (w/v). Conventional laboratory culture method revealed nine (9) bacterial isolates including; Bacillus, Enterobacter, Escherichia, Micrococcus, Proteus, Pseudomonas, Serratia, Staphylococcus, and Streptococcus and two (2) fungal isolates of Candida and Saccharomyces. Whereas, molecular techniques revealed the identity of eleven (11) bacteria including the species of Enterobacter, Escherichia, Klebsiella, Micrococcus, Pseudomonas, Salmonella, Shigella, Staphylococcus, Staphylococcus and Streptococcus andfive (5) fungal genera viz; Candida, Neurospora and Saccharomyce. Staphylococcus aureus, Pseudomonas aeruginos, Candida dubliniensis, Saccharomyces rouxii and Candida albicans were the most frequently isolated organisms from the wound samples. The extracts were found to induce remarkable antimicrobial potential against the test organisms, most especially the hot water and ethanolic extracts with varying ranges of inhibition against the isolates. Proteus vulgaris and Escherichia coliwere most susceptible to 75% of ethanolicextracts, J. curcasLinniat 75% ethanolic root extracts with 12.50±0.00mmand 12.50±0.33mm diameter of the zones of inhibition. Ethanolic leaf extracts of J. curcas Linniat 75% concentration was most effective against P. aeruginosa with 12.00±0.33mm diameter of thezone of inhibition, whereas P. aeruginosa was least susceptible at 75% n-Hexane extracts with 3.50±0.00mmdiameter of the zone of inhibition. Ethanol rated best as the extraction solvent, followed by hot water, n- Hexane and cold water in that order. Generally the antimicrobial potential of the extracts increased with a corresponding increase in extract concentration. Antimicrobial efficacy of the extracts of N. tabacum Linnand J. curcas Linn evaluated in this study had been proven to be well effective andprovided useful information on the importance of N. tabacum Linnand J. curcas Linnas a promising candidate in phytomedicine and to serve as a preventive therapy against the microbial effects in wounds treatment with the view of making it a source ofnatural product or as a basis for the development of new drugs in phytomedicine.

Keywords: *Phytochemicals, sensitivity, phytomedicine, antimicrobial efficacy, jatropha curcas linn and nicotiana tabacum linn*

1. Introduction

Dermal wounds usually with an exposed tissue will naturally become colonized by microorganisms and degenerated tissue will encourage their thriven [Posnett *et al.*,2008.]. Factors and conditions such as the warm, moist, and nutritious conditions usually give more support for the survival and proliferations of the microorganisms colonizing the wounds [Lavery *et al.*, 2006]. Consequently, commensal aerobic and anaerobic micro flora on the human skin is presented with an opportunity to become established in an abnormal but favorable environment, where their survival strategies may render them pathogenic rather than commensal [Bansal*et al.*,2008]. Since microorganisms from a variety of sources are

presented with an opportunity to colonize a common but unnatural habitat, microbial interactions unique to this particular environment may significantly influence wound pathogenesis and cause the delay in the healing process [Hadrys, *et al.,*1992].

Wounds are often polymicrobial, therefore, antimicrobial treatment of clinically infected wounds should cover a variety of potentially synergistic aerobic or facultative and anaerobic microorganisms and should not simply target specific pathogens that are frequently encountered as a causative agent [Francis *et al.,*2005]. Degeneration of a wound to an infected state cannot be correctly diagnosed or assumed by the presence of a specific type of bacterium due to a specific pathophysiological condition, because a multitude of factors are likely to simultaneously influence wound pathogenesis

Fawole, and Oso, 2004.]. Microbiological factors; the types and loads of microorganisms present on the wound and microbial interactions usually contribute immensely to the difficult healing condition of the wound, they are all critical and must be considered collectively as factors predisposing to infection [Bassam, *et al.,*1992]. Clinical studies have demonstrated that a measure of the tissue microbial load in a wound can predict delayed healing or infection [Francis *et al.,*2005][Olutiola et al.,2001] [Openshaw 2000.].

However, on the other hand, any plant of the genus Nicotiana of the Solanaceae family is called tobacco[Bakht *et al.,* 2012]. The tobacco products are manufactured from the leaves, cured & dried, or differs use. The use of tobacco dates back to the ancient civilizations of the Americas,where it played a central role in religious occasions[Sofowora, 2006]. In the 1559, Jean Nicot,the French ambassador to Portugal, wrote about the medicinal properties oftobacco and promoted the usethroughout the world. Because of his great work on tobacco plant, his namewas given to its genus, *Nicotiana*, and its active principle, nicotine[Ezeja*et al.,* 2010]. Ayurveda, folklorepractices and traditional aspects of therapeutically important natural productstobacco one of them. Tobacco is processed from the leaves of plants in thegenus i.e. *Nicotiana*. Nicotine tartrate used as a pesticide as well as inmedicines. It is commonly used as a cash crop in countries like India, China,Cuba and the United States[Sofowora, 2006].

Tobacco plants are also used in bioengineering and as ornamentals plant. The pharmacological activities of *N. tabacum* is mostly due to its content of nicotine which stimulates the nicotine receptors leading to release of substances such as acetylcholine, nor-epinephrine, dopamine, serotonin, vasopressin and growth hormone[Ezeja*et al.,* 2010]. Nicotine is a major component of tobacco has been demonstrated to accelerate angiogenesis and wound healing in genetically diabetic mice. The ethno medical uses include theuse of the decoction of leaves as antispasmodics, diuretics, emetics, expectorants, sedatives, and in rheumatic swellings, anesthetics, antibacterial, antimicrobial, an the lmintic, anticonvulsants and for anti-fungal activities[Bakht *et al.,* 2012]. Moreover, *Jatropha curcas Linn*is a perennial plant belonging to family euphorbiaceae [Openshaw, 2000]. *Jatropha* plants can easily be grown and usually cultivated as biodiesel crop in many tropical and sub-tropical countries and can also be seen growing as fence around crop plants in many regions of India[Francis*et al.,* 2005].Growth of *Jatropha* plants on saline soils and their potential for accumulating sodium, potassium and chloride are the attributes suggesting the possibility of use of *Jatropha* plants in improving saline soils[Francis*et al.,* 2005]. Non-edible oil produced from the seeds is used as feed stock for the production of bio-diesel. Press cake is used to improve soil and for the production of biogas [Gübitz *et al.,* 1999]. It is a plant that produces a wide range of bioactive compounds, some of which are allelopathic in nature. *J. curcas Linn* has an invasive characters and is a significant source of many phytochemicals with varying biological activities [Muangman*et al.,* 2005]. Different plant parts of *J. curcasLinn* exhibited variation in their phytochemical constituents. Leaves and floral parts were found to contain higher contents of total phenols, tannins and phytic acid[Mujumdar*et al.,* 2001].

The purpose of the present study was to determine the antimicrobial efficacy of different parts of *N. tabacumLinn*and *J. curcas Linn*extracts against microbes colonizing the wounds of diabetic patients in view to evaluate theirantimicrobial sensitivity profile and compared with that of typed cultures using disc diffusion method. Thereafter, the antimicrobial efficacy of the plant extracts against isolates that showed multiple antibiotic resistance was determined using agar well diffusion technique [Olutiola *et al.,* 2001]. At present, microbiology of wounds has been actively researched [Lipsky*et al.,* 2012], yet the microbial mechanisms that induce infection and prevent wound healing had not been adequately exploit. Consequently, debate regarding microbial involvement in wound healing is likely to persist [Gottrup, 2004]. Natural bioactive compounds have shown various anti-bacterial, anti-fungal, and anti-inflammatory properties [Amarowicz, 2007]. They are gaining considerable attention as eco-friendly alternative to synthetic antimicrobial active compound [Sofowora, 2006].

2. Materials and Methods

2.1. Collection of Clinical Sample

A total of 454 (Four hundred and fifty four) clinical swab samples were collected from the wounds of diabetic patients in Ondo state; Federal Medical Center Owo, Ondo State Specialist hospital Akure, Gani-fawehinmi Diagnostic center Ondo, Ikare and Okitipupa General Hospitals, between April, 2016 and December, 2016 within the hour of 8am and 10am daily. Sterile swab sticks were used for clinical sample collection and transported in ice bath to the laboratory for analysis within 1h of collection for isolation [Cheesbrough, 2010], characterization and antimicrobial assay culturing using suitable culture media such as Nutrient Agar, Chocolate Agar, Sabouraud Dextrose Agar, Eosin Methylene Blue Agar and Mueller Hinton Agar [Fawole, and Oso, 2004].

2.2. Isolation and Identification of Clinical Sample

The isolates were characterized and identified as described by Bergey's manual [Bergey*et al.,* 1994] [Cheesbrough, 2010].

2.3. Characterization of Isolates

The isolates were identified and characterized according to Cheesebough (2010) on the basis of colonial morphology, microscopy (Gram staining reaction), sugar fermentation/utilization and other metabolic biochemical reaction testing. The isolates were further identified with reference to Bergey's manual of determinative bacteriology [Bergey*et al.,* 1994] [Cheesbrough, 2010].

2.4. Extraction of DNA using CTAB (CetyItrimethyl Ammonium Bromide) Method

Microbial isolates were grown overnight and it was transferred to eppendorf tube and spun down at 14,000rpm for 2mins, the supernatant was discarded and 600µl of 2X CTAB buffer was added to the pellet and it was incubated at 65^{o}C for 30mins. The sample was removed from the incubator and allowed to cool to room temperature and chloroform was added, the sample was mixed by gently inversion of the tube several times. Thereafter, the sample was spun at 14,000rpm for 15mins and the supernatant was transferred into a new eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1hr and later spun at 14,000rpm for 10mins and the supernatant was discarded and the pellet was washed with 70% ethanol later the sample was air dried for 30mins on the bench. The pellet was resuspended in 100ul of sterile distilled water. DNA concentration of the samples was measured on spectrophotometer at 260nm and 280nm and the genomic purity were determined. The genomic purity was between 1.8 –2.0 for all the DNA samples [Melendez, 2010].

2.5. DNA Electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gel. Agarose gel was prepared by dissolving and boiling 1.0g agarose in 100ml 0.5 X TBE buffer solutions. The gels were allowed to cool down to about 45^{o}c and 10ul of 5mg/ml ethidium bromide were added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3ul of the DNA with 5ul sterile distilled water and 2ul of 6X loading dye were mixed together and loaded in the well created. Electrophoresis was done at 80V for 2hrs. The integrity of the DNA were visualized and photographed on UV light source [Williams*etal*., 1990][Bassam, *et al.,* 1992].

2.6. PCR Analysis Using ITS1 and ITS4 Primers

PCR analysis was run with a universal primer for fungi called 1TS1 and ITS4. The PCR mix comprises of 1µl of 10X buffer, 0.4µl of 50mM $MgCl_2$, 0.5µl of 2.5mMdNTPs, 0.5µl 5mM ITS1 primer, 0.5µl of 5mM ITS4 primer, 0.05µl of 5units/µlTaq with 2µl of template DNA and 5.05µl of distilled water to make-up 10µl reaction mix.The PCR profile used is initial denaturation temperature of 94^{o}C for 3mins, followed by 30 cycles of 94^{o}C for 60sec, 56^{o}C for 60sec, 72^{o}C for 120sec and the final extension temperature of 72^{o}C for 5mins and the 10^{o}C hold forever [Davies, 2004].

2.7. Purification of PCR Products

The amplicon is further purified before the sequencing using 2M Sodium Acetate wash techniques. To about 10µl of the PCR product, add 1µl 2M NaAct pH 5.2, followed by 20µl Absolute Ethanol, keep at -20^{o}C for 1hr, spin at 10,000rpm for 10mins, then wash with 70% ethanol and air dried. Resuspended in 5µl sterile distilled water and keep at 4^{o}C for sequencing [11].

2.8. PCR for Sequencing

The primers used for the reaction are ITS1 and ITS4. The PCR mix used includes 0.5µl of BigDye Terminator Mix,1µl of 5X sequencing buffer, 1µl of M13 forward primer with 6.5µl Distilled water and 1µl of the PCR product making a total of 10µl. The PCR profile for Sequencing is a Rapid profile, the initial Rapid thermal ramp to 96^{o}C for 1min followed by 25 cycles of Rapid thermal ramp to 96^{o}C for 10 seconds Rapid thermal ramp to 50^{o}C for 5 seconds and Rapid thermal ramp to 60^{o}C for 4 minutes, then followed by Rapid thermal ramp to 4^{o}C and hold forever [19].

2.9. Preparation of Sample for Gene Sequencer (ABI 3130xl Machine)

The Cocktail mix is a combination of 9µl of Hi di Formamide with 1µl of Purified sequence making a total of 10µl. The samples were loaded on the machine and the data in form A, C, T, and G will be released [Williams *etal*., 1990].

2.10. Collection of Plant Samples

Two different medicinal plants namely; *N. tabacum* and *J. curcas* were collected from some farmlands in Ondo state, between May and October, 2016. The farmlands were located at; F.U.T.A. farm garden, Igbatoro farm settlement (Akure south-Ondo central), Bolorunduro farm settlement (in Ondo-East), Idanre thick forest, Iju/Itaogbolu farm settlement and Ilaramokin farm settlement. Different parts of plants were separately collected for process, the parts are; Root, Leaf, Stem, Seed and Shaft from each of the plant and were identified using standard monograph and traditional method of identification. The plant samples were further authenticated at the International Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria.

2.11. Processing of Plant Extracts

The plant' parts were cleaned and shade air-dried for 5 weeks at room temperature (25^{o}C) and then ground to powder with a mechanical grinder (Thomas Wiley machine, model 5 USA). Powders (200gs) of each plant were extracted

with 1litre of sterile aqueous water (both ordinary cold water and hot water at 95^{o}C), ethanol and normal Hexane separately at room temperature (25^{o}C). They were labeled as crude extracts and kept in the universal bottle for further use [Fawole, and Oso, 2004][Bakht *et al.,* 2012].

2.12. Determination of Percentage Yield of the Plant Extracts

The crude plant extracts were filtered with sterile double layered muslin cloth and re-filtered using Whatman's No 1 filter paper with pore size of 110 mm. The high polar solvents (ethanol) extracts were concentrated at 45^{o}C using a rotary evaporator (RE -52 A Union Laboratories, England), while the water extracts were evaporated at 50^{o}C in a water bath[Chees brough, 2010][Clinical and Laboratory Standards Institute, 2010]. The percentage yield of the extracts was calculated thus:

$$\frac{\text{Weight of extract recovered after concentration}}{\text{Initial weight of dried-powdered plant sample}} \times 100$$

2.13. Preparation of Plant Extracts

A 150g portion of the powdered sample was soaked in 750ml in each of ethanol, n-Hexane, cold water (10% chloroform water) and hot water (95^{o}c) in a conical flask. The flask was shaken properly for 2mins and then allowed to stand for 72h. Thereafter, the mixture was filtered through Whatman No 1 filter paper and the filtrate was evaporated in vacuo using Buchi Rota vapor R-14. The rotary flask was rinsed with part of the solvent and the solution was poured in a specimen bottle. Thereafter, the bottle was kept in the hood for the solvent to evaporate, leaving the dried extract in the specimen bottle. The dried plant extracts was then reconstituted using 30ml of 20% Tween-20 (Polysorbate 20) [Olutiola *et al.,* 2001] [Fawole, and Oso, 2004].

2.14. Preparation of Different Concentrations of the Extracts

Each extract was diluted with 20% Tween-20 to obtain different concentrations of 25%, 50%, 75% and 100%. The undiluted extract was taken as 100% extract concentration. A 1g of portion of the reconstituted extract was dissolved in 1ml of 20% Tween-20, for 75% extract concentration; then 0.75g of reconstituted extract was dissolved in 0.25ml of 20% Tween-20, for 50% extract concentration; A 0.50g of reconstituted extract was dissolved in 0.50ml of 20% Tween-20 and for 25% extract concentration; A 0.25g portion of reconstituted extract was dissolved in 0.75ml of 20% Tween-20 [Clinical and Laboratory Standards Institute, 2010].

2.15. Antibacterial Assay

The antimicrobial activity of the extracts was carried out using disc diffusion methods [Fawole, and Oso, 2004]. A day old bacterial colonies (3-5) obtained from a standardized inoculum on the nutrient agar culture media was transferred into a sterile normal saline that make up to1.5×10^{8} cfu/ml. With the use of sterile forceps, the extracts discs was placed on the surface of the agar culture media seeded with each microorganism and spread with the use of sterile glass spreader, incubate at 37 °C for 24h. The zones of inhibition was taken by measuring with the use of standard vernier calliper considering the total diameters of the zone of inhibition and the same procedure applied to each plate that serve as a control using 0.3ml of 20% Tween-20 which was used as the reconstituting solvent[Olutiola *et al.,* 2001]. The tests were carried out in triplicates, allowed to stand at room temperature for about 2h and then incubated. The diameter of the zone of inhibition was measured in millimetres afterwards. The assay for those extracts impregnated discs that shows inhibition against microbial growth was further evaluated based on the values that were taken as their zone of inhibition[Clinical and Laboratory Standards Institute, 2010].

2.16. Antifungal Assay

The antimicrobial activity of the extracts was carried out using disc diffusion methods [Fawole, and Oso, 2004] [Olutiola *et al.,* 2001]. A 2-day old fungal culture on the Sabouraud-dextrose culture media was transferred into a sterile saline that was made up to 1.5×10^{6} sfu/ml. With the use of sterile forceps, the extracts incorporated discs were placed on the surface of the agar culture media containing each microorganism spread with the use of sterile glass spreader. The plates were then incubated at 25ºC for 48h. The zones of inhibition were taken by measuring with the use of standard vernier calliper considering the total diameters of the zone of inhibition. The same procedure was applied to each plate that represent the control using0.3ml of 20% Tween-20 which was used as the reconstituting solventcorresponding isolates [Clinical and Laboratory Standards Institute, 2010]. The tests were carried out in triplicates, allowed to stand at room temperature for about 2h and then incubated; the diameter of the zone of inhibition was measured in millimetre.The antifungal studies involved the use of the extracts of the plant by employing the agar well diffusion method. Aliquots of spore were prepared by mixing loopful of fungal spores in sterile distilled water. Spore suspension (0.3ml) was put into a sterile petri dish, prepared potato dextrose agar was then poured into the plate, swirled and allowed to solidify. A sterile cork borer (6mm diameter) was used to create 3mm depth of wells inside the culture plates. The crude extracts were reconstituted with 20% Tween-20 and introduced into the well[Fawole, and Oso, 2004][Clinical and Laboratory Standards Institute, 2010].

2.17. Data Analysis

Data were presented as mean ± standard error (SE), and subjected to two-way analysis of variance (ANOVA). Treatment means were compared using Duncan's New Multiple Range Tests (DNMRT) at $P \leq 0.05$ level of significance with computer aided Statistical Package for Social Sciences (SPSS) version 17.

3. Results and Discussion

3.1. Clinical Isolates Identity and their Biochemical Characteristics

With conventional laboratory culture methods followed by the biochemical tests on the pure culture cultural methods; Conventional laboratory culture method revealed nine (9) bacterial isolates including; *Bacillus, Enterobacter, Escherichia, Micrococcus, Proteus, Pseudomonas, Serratia, Staphylococcus,* and *Streptococcus* and two (2) fungal isolates of *Candida* and *Saccharomyces.* Thereafter, using molecular technique for the identity of the isolates, the following bacteria were detected; *Staphylococcus epidermidis, Salmonella typhil, Micrococcus luteus, Streptococcus pyogenes, Staphylococcus aureus, Enterobacter cloacae, Pseudomonas aeruginosa, Escherichia coli, Shigella flexineri, Klebsiella pneumonia, Pseudomonas Putidae*(Table 1),and among the fungi were; *Neurospora crassa, Candida dubliniensis, Candida albican, Saccharomyces cerevisiae* and *Saccharomyces rouxii*(Table 2).Total number of eleven (11) bacteria and five (5) fungi were identified using molecular technique. The use of molecular technique is more sensitive, reliable, adequate, and accurate which can identifies more microorganism than limiting to the cultural technique in microbial type-determination and load [Devarshi and Gajjar, 2013][Bassam, *et al.,* 1992][Melendez, 2010].

3.2. Percentage Yield in Solvent Extraction of the Plant Extract

The percentage yield of the extracts of different parts of the plants (*J. curcas* and *N. tabacum*) in various solvent (Ethanol, normal hexane, hot water and ordinary cold water) shows there is an highest yield using ethanol solvent and hot water while the stem, leaf and root of the plants yielded more considerable percentage quantity of extract (Table 3) compared to other parts of the both plants [Bakht *et al.,* 2012].

Results: Comparison of antimicrobial activities of different parts of N. tabacum and J. curcas extracts

Isolate/microorganism identified	Morphology characterization/shape	Motility test	Gram staining reaction	Catalase reaction	Oxidase reaction	Coagulase reaction	Indole reaction	Methyi Red reaction	Vorges proskaurer test	Glucose	Maltose	Sucrose	Lactose	Urase test	Nitrate reduction	H_2S production	oxidation/fermentation test	Microbial count/size(CFU)	Turbidity	Gaseous requirement
Bacteria																				
Micrococcus luteus	Ova	+	+	+	-	+	-	+	-	+	+	+	+		-		-	$1.8x10^6$	-	Obligate aerobes
Streptococcus pyogenes	Spiril	+	+	+	-	+	-	+	+or-	+	+	+	+		-	-	-	$1.2x10^6$	+	Facultative (aerotolerant) anaerobes
Staphylococcus aureus	Round	+	+	+		+	-	+		+	+	+	+		+		-	$1.0x10^6$	+	Aerobes
Staphylococcus epidermidis	Round	+	+	+	-	+	-	+		+	+	+	+		+		-	$1.3x10^6$	+	Aerobes
Enterobacter cloacae	Cocci	+	+	+		-	+	+	-				+		+	-		$1.8x10^6$	+	Aerobes

Isolate/microorganism identified	Morphology characterization/shape	Motility test	Gram staining reaction	Catalase reaction	Oxidase reaction	Coagulase reaction	Indole reaction	Methyi Red reaction	Vorges proskaurer test	Glucose	Maltose	Sucrose	Lactose	Urase test	Nitrate reduction	H_2S production	oxidation/ferment	Microbial count/size(CFU)	Turbidity	Gaseous requirement
Pseudomonas aeruginosa	Rod	+	-	-	+	-	+	-	+	-	-	-	-		+	-	+	1.8x 10^6	+	Aerobes
Salmonella typhil	Rod	+	-	-	-	-	-		-	-	-	-	-		+	+		2.5x 10^6	+	Aerobes
Escherichia coli	Rod	-	-	+		-	+	+	-				+	-	+	-	-	2.3x 10^6	+	Aerobes
Shigella flexineri	Cocci	-	-	+	-		+	-	+		-		-		+	-		2.1x 10^6	+	Aerobes
Klebsiella pneumonia	Rod	-	-		-	-	+	- or +	+		+		+		+	-		3.0x 10^6	+	Aerobes
Pseudomonas Putidae	Rod	-	-	-	+	-	+	-	+	-	-	-	-		+			1.8x 10^6	+	Aerobes

Table 1: Biochemical Characteristics of the Bacterial Isolates

Isolate/microorganism identified	Morphology characterization/shape	Motility test	Lactophenol in cotton blue stain	Catalase reaction	Oxidase reaction	Coagulase reaction	Indole reaction	Methyi Red reaction	Vorges proskaurer test	Glucose	Maltose	Sucrose	Lactose	Urase test	Nitrate reduction	H_2S production	oxidation/fermentation	Microbial count/size(SFU)	Turbidity	Gaseous requirement
Fungi																				
Saccharomyces cerevisiae	Yeast/ova	viscous	Purple															0.8X10^3	+	Facultative anaerobes
Neurospora crassa	Budding		Purple															1.0X10^3	+	Facultative anaerobes
Candida albican	Ova		Purple															1.3X10^3	+	Facultative anaerobes
Candida dubliniensis	Ova/budding	Mucoid	Purple															1.8X10^3	+	Facultative anaerobes
Saccharomyces rouxii	Yeast	Mucoid	Purple															1.0X10^3	+	Facultative anaerobes

Table 2: Colonial Morphological and Microscopic Characteristics of Fungal Isolates

Solvent	Stem	Leaf	Seed	Root	Stem	Leaf	Seed	Root
Ordinary Water	15	22	9	18	11	25	8	19
Hot Water	13	18	11	22	13	20	11	15
Normal –Hexane	12	15	12	19	15	15	15	20
Ethanol	25	29	15	25	22	27	17	27

Table 3: Yield of the Extract (%) J. curcas N. tabacum

	Water		% of Ethanolic Solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Bacteria)											
Micrococcus luteus	7.00 ± 0.53^{bc}	8.52 ± 0.00^{b}	5.50 ± 0.33^{bc}	6.50 ± 0.33^{bc}	11.50 ± 0.33^{a}	12.00 ± 0.62^{b}	4.50 ± 0.57^{b}	5.00 ± 0.00^{ab}	6.50 ± 0.00^{ac}	7.00 ± 0.62^{b}	0.00 ± 0.00^{ab}
Streptococcus pyogenes	6.50 ± 0.33^{b}	5.23 ± 0.00^{b}	3.50 ± 0.33^{a}	5.00 ± 0.00^{b}	12.00 ± 0.62^{b}	14.00 ± 0.62^{b}	3.50 ± 0.33^{a}	5.90 ± 0.57^{bc}	6.00 ± 0.57^{ac}	5.50 ± 0.00^{b}	0.00 ± 0.00^{a}
S. aureus	8.00 ± 0.67^{b}	9.50 ± 0.62^{b}	5.00 ± 0.62^{b}	6.50 ± 0.25^{b}	9.50 ± 0.00^{b}	11.20 ± 0.00^{b}	5.50 ± 0.33^{bc}	6.50 ± 0.25^{b}	7.00 ± 0.00^{a}	6.50 ± 0.25^{b}	0.00 ± 0.00^{a}
Staphylococcus epidermidis	6.00 ± 0.42^{ab}	8.00 ± 0.33^{bc}	5.50 ± 0.00^{b}	7.00 ± 0.00^{bc}	10.00 ± 0.00^{bc}	11.20 ± 0.00^{b}	5.20 ± 0.00^{bc}	6.50 ± 0.62^{b}	6.20 ± 0.62^{b}	9.50 ± 0.62^{b}	0.00 ± 0.00^{ac}
Enterobacter cloacae	5.23 ± 0.00^{b}	7.50 ± 0.57^{ac}	4.20 ± 0.00^{b}	4.50 ± 0.57^{b}	11.50 ± 0.33^{a}	13.5 ± 0.25^{b}	4.20 ± 0.00^{b}	7.00 ± 0.00^{b}	6.50 ± 0.57^{b}	7.00 ± 0.00^{bc}	0.00 ± 0.00^{ac}
Pseudomonas aeruginosa	4.67 ± 0.33^{bc}	8.50 ± 0.00^{ac}	0.00 ± 0.00^{ab}	5.20 ± 0.00^{b}	9.00 ± 0.00^{b}	12.50 ± 0.57^{b}	5.00 ± 0.33^{bc}	5.00 ± 0.57^{bc}	4.50 ± 0.57^{b}	5.00 ± 0.33^{bc}	0.00 ± 0.00^{ab}
Salmonella tphyil	5.00 ± 0.33^{bc}	6.90 ± 0.57^{bc}	4.00 ± 0.00^{ab}	4.50 ± 0.57^{b}	9.50 ± 0.57^{b}	10.50 ± 0.00^{ac}	4.00 ± 0.62^{ab}	5.50 ± 0.57^{b}	5.50 ± 0.33^{bc}	3.50 ± 0.33^{a}	0.00 ± 0.00^{a}
Escherichia coli	8.20 ± 0.00^{b}	9.00 ± 0.57^{a}	5.20 ± 0.00^{bc}	6.00 ± 0.57^{bc}	9.50 ± 0.00^{b}	11.5 ± 0.57^{a}	5.50 ± 0.00^{b}	7.00 ± 0.00^{b}	6.00 ± 0.00^{bc}	5.00 ± 0.62^{b}	0.00 ± 0.00^{ab}
Shigella flexineri	6.50 ± 0.62^{b}	8.50 ± 0.62^{ab}	5.50 ± 0.33^{bc}	6.50 ± 0.33^{bc}	10.5 ± 0.25^{b}	9.00 ± 0.00^{bc}	6.50 ± 0.33^{bc}	6.00 ± 0.25^{b}	6.20 ± 0.25^{b}	5.00 ± 0.57^{b}	0.00 ± 0.00^{a}

	Water		% of Ethanolic Solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Klebsiella pneumonia	5.00 ± 0.57^{b}	11.5 ± 0.57^{a}	4.00 ± 0.62^{ab}	7.00 ± 0.62^{b}	11.20 ± 0.00^{b}	10.00 ± 0.00^{bc}	5.00 ± 0.57^{bc}	7.00 ± 0.00^{b}	5.00 ± 0.00^{b}	4.20 ± 0.00^{b}	0.00 ± 0.00^{ab}
Pseudomonas putidae	5.50 ± 0.33^{bc}	7.20 ± 0.00^{a}	5.00 ± 0.57^{a}	5.20 ± 0.00^{bc}	11.50 ± 0.57^{a}	8.50 ± 0.62^{ab}	6.50 ± 0.00^{ac}	4.50 ± 0.62^{bc}	5.00 ± 0.00^{ac}	3.50 ± 0.33^{a}	0.00 ± 0.00^{b}
Test Organisms (Fungi)											
Saccharomyces cerevisiae	4.20 ± 0.00^{b}	7.00 ± 0.62^{b}	0.00 ± 0.00^{a}	4.50 ± 0.57^{b}	7.00 ± 0.62^{bc}	5.23 ± 0.00^{b}	2.50 ± 0.00^{ac}	4.00 ± 0.00^{a}	4.50 ± 0.57^{ac}	4.50 ± 0.57^{b}	0.00 ± 0.00^{bc}
Neurospora crassa	5.00 ± 0.62^{b}	6.50 ± 0.00^{b}	4.20 ± 0.00^{b}	5.50 ± 0.25^{b}	6.50 ± 0.00^{ac}	5.23 ± 0.00^{b}	3.50 ± 0.33^{a}	4.50 ± 0.62^{bc}	5.00 ± 0.57^{a}	3.50 ± 0.57^{b}	0.00 ± 0.00^{b}
Candida albican	5.50 ± 0.00^{b}	5.50 ± 0.33^{bc}	4.00 ± 0.00^{a}	5.00 ± 0.57^{bc}	7.50 ± 0.00^{ac}	8.52 ± 0.00^{b}	0.00 ± 0.00^{a}	5.00 ± 0.57^{bc}	4.00 ± 0.62^{ab}	2.50 ± 0.62^{ab}	0.00 ± 0.00^{ab}
Candida dubliniensis	3.50 ± 0.33^{a}	7.00 ± 0.62^{b}	0.00 ± 0.00^{bc}	4.20 ± 0.57^{b}	5.50 ± 0.62^{a}	5.23 ± 0.00^{b}	1.00 ± 0.00^{a}	4.50 ± 0.57^{ac}	5.50 ± 0.62^{ab}	4.67 ± 0.33^{bc}	0.00 ± 0.00^{a}
Saccharomyces rouxii	5.00 ± 0.57^{b}	6.00 ± 0.33^{a}	3.50 ± 0.33^{a}	5.00 ± 0.00^{b}	5.00 ± 0.00^{a}	6.00 ± 0.42^{ab}	3.00 ± 0.00^{a}	5.20 ± 0.00^{bc}	4.00 ± 0.00^{ab}	3.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Table 4: Antimicrobial Sensitivity Pattern of the Tested Microorganisms against Extracts.
(Zone Of Inhibition Measured In Millimeter 'Mm')
Data Are Presented as Mean±S.E. Value with the Same Superscript Along the Column Are Not Significantly Different (P≤0.05).

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Bacteria)											
Micrococcus luteus	5.00±0.57^{a}	6.50±0.62^{b}	3.50±0.62^{bc}	5.00±0.62^{bc}	8.50±0.57^{b}	7.00±0.57^{a}	4.50±0.62^{a}	5.00±0.57^{a}	6.00±0.00^{ac}	5.00±0.00^{ab}	0.00±0.00^{a}
Streptococcus pyogenes	5.50±0.57^{ac}	7.00±0.57^{b}	5.50±0.57^{ac}	5.00±0.62^{a}	10.00±0.00^{b}	9.50±0.00^{b}	0.00±0.00^{ab}	5.50±0.57^{a}	6.00±0.57^{ac}	6.50±0.57^{bc}	0.00±0.00^{a}
S. aureus	7.00±0.00^{ac}	8.50±0.62^{bc}	4.50±0.62^{a}	5.50±0.57^{a}	7.59±0.57^{b}	4.50±0.57^{b}	3.50±0.62^{bc}	6.50±0.00^{a}	6.50±0.00^{b}	7.50±0.57^{ac}	0.00±0.00^{ac}
S. epidermidis	5.20±0.62^{bc}	7.00±0.00^{ac}	4.59±0.57^{b}	3.50±0.62^{a}	9.67±0.00^{b}	10.00±0.00^{ab}	5.00±0.57^{a}	5.50±0.00^{ab}	6.50±0.00^{b}	6.90±0.00^{ac}	0.00±0.00^{ac}
Enterobacter cloacae	6.00±0.00^{a}	7.00±0.00^{a}	2.50±0.62^{b}	4.50±0.57^{a}	9.50±0.00^{b}	11.00±0.00^{a}	3.00±0.33^{bc}	7.00±0.57^{a}	5.50±0.25^{b}	6.50±0.00^{a}	0.00±0.00^{ab}
P. aeruginosa	3.50±0.62^{bc}	7.50±0.62^{ab}	4.50±0.57^{b}	5.00±0.00^{ab}	8.00±0.33^{a}	7.00±0.57^{b}	5.00±0.00^{ab}	5.00±0.57^{a}	4.50±0.57^{b}	4.59±0.57^{b}	0.00±0.00^{a}
Salmonella tphyil	4.50±0.62^{a}	6.90±0.00^{ac}	3.00±0.00^{b}	4.50±0.57^{bc}	8.00±0.62^{b}	9.50±0.57^{bc}	4.50±0.62^{a}	5.00±0.33^{b}	5.00±0.62^{b}	7.00±0.00^{a}	0.00±0.00^{ab}
Escherichia coli	6.50±0.57^{ac}	7.50±0.57^{ac}	5.20±0.62^{bc}	6.00±0.00^{ac}	7.50±0.57^{bc}	8.00±0.33^{bc}	6.00±0.00^{ac}	7.00±0.00^{b}	6.00±0.00^{b}	8.00±0.33^{a}	0.00±0.00^{a}
Shigella flexineri	5.00±0.00^{ac}	8.00±0.25^{b}	5.00±0.57^{a}	6.50±0.00^{a}	9.50±0.62^{ab}	10.50±0.62^{b}	4.59±0.57^{b}	6.50±0.33^{bc}	5.50±0.62^{b}	4.00±0.33^{bc}	0.00±0.00^{ab}
Klebsiella pneumonia	4.50±0.62^{a}	10.50±0.62^{b}	4.50±0.62^{b}	6.00±0.33^{a}	10.00±0.00^{ab}	11.00±0.33^{bc}	3.50±0.62^{bc}	5.00±0.62^{b}	5.00±0.25^{b}	3.00±0.00^{b}	0.00±0.00^{b}
Pseudomonas putidae	5.00±0.00^{b}	6.50±0.57^{bc}	3.00±0.33^{bc}	4.50±0.57^{bc}	11.0±0.00^{ac}	6.50±0.57^{bc}	2.50±0.62^{b}	4.50±0.62^{b}	5.00±0.00^{b}	4.50±0.57^{a}	0.00±0.00^{ab}

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	Hot	Ordinary	25	50	75	100	25	50	75	100	20%Tween 20
Test Organisms (Fungi)											
S. cerevisiae	4.59 ± 0.57^{b}	7.00 ± 0.33^{ab}	2.00 ± 0.33^{bc}	4.00 ± 0.33^{bc}	7.00 ± 0.62^{bc}	6.50 ± 0.62^{a}	4.50 ± 0.62^{a}	5.00 ± 0.33^{bc}	4.50 ± 0.57^{b}	5.00 ± 0.00^{ab}	0.00 ± 0.00^{ab}
Neurospora crassa	5.00 ± 0.00^{bc}	6.50 ± 0.00^{b}	3.50 ± 0.62^{bc}	7.00 ± 0.00^{b}	6.00 ± 0.00^{a}	7.50 ± 0.57^{ac}	5.00 ± 0.57^{a}	4.50 ± 0.57^{b}	6.00 ± 0.25^{b}	4.50 ± 0.57^{b}	0.00 ± 0.00^{a}
Candida albican	5.00 ± 0.33^{b}	6.50 ± 0.33^{b}	4.50 ± 0.62^{a}	6.00 ± 0.33^{ab}	6.50 ± 0.00^{ac}	2.50 ± 0.62^{b}	3.00 ± 0.33^{bc}	5.50 ± 0.33^{ab}	5.00 ± 0.00^{a}	4.59 ± 0.57^{b}	0.00 ± 0.00^{a}
Candida dubliniensis	4.00 ± 0.62^{b}	7.00 ± 0.00^{bc}	4.00 ± 0.62^{b}	5.50 ± 0.62^{b}	5.00 ± 0.57^{ac}	5.00 ± 0.00^{b}	5.20 ± 0.62^{bc}	6.00 ± 0.00^{b}	5.50 ± 0.33^{bc}	6.00 ± 0.00^{ac}	0.00 ± 0.00^{ac}
Saccharomyces rouxii	4.50 ± 0.57^{bc}	6.0 ± 0.33^{bc}	4.50 ± 0.62^{b}	5.69 ± 0.25^{b}	5.50 ± 0.62^{bc}	4.50 ± 0.62^{a}	0.00 ± 0.00^{ac}	5020 ± 0.25^{b}	4.00 ± 0.62^{b}	4.50 ± 0.62^{a}	0.00 ± 0.00^{ac}

Table 5: Antimicrobial Sensitivity Pattern of the Tested Microorganisms Againstextracts.
(Zone of Inhibition Measured In Millimeter 'Mm')
Root Extracts of N. Tabacum in Different Solvents Data Are Presented as Mean±S.E. Value with the Same Superscript along the Column Are Not Significantly Different (P≤0.05)

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Bacteria)											
Micrococcus luteus	6.50 ± 0.00^{bc}	10.00 ± 0.00^{a}	5.50 ± 0.62^{bc}	7.00 ± 0.00^{bc}	10.50 ± 0.00^{a}	8.00 ± 0.57^{b}	4.00 ± 0.57^{bc}	4.50 ± 0.57^{bc}	6.50 ± 0.57^{a}	5.50 ± 0.62^{bc}	0.00 ± 0.00^{ab}
Streptococcus pyogenes	6.00 ± 0.62^{b}	9.00 ± 0.57^{a}	3.50 ± 0.00^{ac}	5.00 ± 0.57^{b}	9.50 ± 0.57^{ac}	6.00 ± 0.62^{b}	0.00 ± 0.00^{ac}	5.50 ± 0.62^{b}	6.00 ± 0.57^{a}	8.00 ± 0.57^{ac}	0.00 ± 0.00^{a}
S. aureus	4.00 ± 0.57^{bc}	12.50 ± 0.62^{ab}	4.00 ± 0.57^{bc}	7.00 ± 0.33^{a}	12.00 ± 0.00^{ac}	9.50 ± 0.33^{bc}	4.50 ± 0.33^{a}	5.00 ± 0.00^{b}	5.50 ± 0.00^{ab}	5.50 ± 0.25^{b}	0.00 ± 0.00^{a}
S. epidermidis	5.50 ± 0.25^{b}	8.00 ± 0.57^{ac}	5.50 ± 0.62^{bc}	5.50 ± 0.33^{bc}	8.00 ± 0.00^{a}	8.00 ± 0.57^{ac}	5.59 ± 0.62^{bc}	6.50 ± 0.62^{b}	4.50 ± 0.62^{a}	6.00 ± 0.62^{b}	0.00 ± 0.00^{ac}
Enterobacter cloacae	6.00 ± 0.57^{bc}	7.50 ± 0.62^{a}	4.50 ± 0.62^{a}	6.00 ± 0.57^{bc}	9.00 ± 0.62^{bc}	9.50 ± 0.33^{bc}	4.50 ± 0.33^{a}	5.50 ± 0.33^{b}	6.50 ± 0.62^{ab}	6.00 ± 0.62^{b}	0.00 ± 0.00^{ac}
P. aeruginosa	6.00 ± 0.62^{bc}	9.50 ± 0.33^{bc}	6.50 ± 0.62^{a}	7.50 ± 0.00^{b}	10.50 ± 0.00^{a}	6.00 ± 0.62^{b}	0.00 ± 0.00^{ab}	6.50 ± 0.57^{b}	6.50 ± 0.00^{ac}	4.50 ± 0.62^{a}	0.00 ± 0.00^{ab}
Salmonella tphyil	5.50 ± 0.00^{ab}	8.00 ± 0.00^{b}	6.00 ± 0.62^{b}	6.50 ± 0.25^{b}	9.50 ± 0.57^{a}	7.00 ± 0.33^{a}	5.00 ± 0.57^{ac}	5.00 ± 0.00^{b}	5.50 ± 0.00^{ac}	6.50 ± 0.57^{bc}	0.00 ± 0.00^{a}
Escherichia coli	5.50 ± 0.62^{bc}	8.00 ± 0.57^{b}	5.00 ± 0.57^{ac}	5.00 ± 0.62^{b}	7.50 ± 0.33^{a}	12.50 ± 0.62^{ab}	3.50 ± 0.00^{ac}	6.00 ± 0.25^{b}	6.50 ± 0.33^{b}	5.00 ± 0.57^{ac}	0.00 ± 0.00^{ab}
Shigella flexineri	6.50 ± 0.62^{a}	8.50 ± 0.25^{b}	4.00 ± 0.57^{bc}	6.50 ± 0.57^{bc}	8.50 ± 0.00^{b}	5.50 ± 0.00^{ab}	0.00 ± 0.00^{b}	4.50 ± 0.33^{a}	7.00 ± 0.00^{b}	5.50 ± 0.62^{a}	0.00 ± 0.00^{a}

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Klebsiella pneumonia	5.59±0.62^{bc}	10.00±0.25^{b}	0.00±0.00^{ab}	6.00±0.62^{b}	9.50±0.33^{ab}	8.00±0.57^{ac}	5.59±0.62^{bc}	5.50±0.25^{b}	6.00±0.33^{bc}	3.50±0.00^{ac}	0.00±0.00^{ab}
Pseudomonas putidae	5.00±0.57^{ac}	10.50±0.57^{b}	4.50±0.33^{a}	8.50±0.00^{ab}	8.00±0.33^{b}	4.50±0.62^{a}	4.00±0.57^{bc}	4.50±0.00^{bc}	5.50±0.62^{b}	6.00±0.00^{b}	0.00±0.00^{b}
Test Organisms (Fungi)											
S. cerevisiae	3.20±0.62^{a}	6.50±0.33^{bc}	3.20±0.62^{a}	4.50±0.62^{a}	4.50±0.62^{b}	8.00±0.57^{ac}	0.00±0.00^{ab}	4.00±0.57^{bc}	5.50±0.57^{b}	7.50±0.62^{a}	0.00±0.00^{bc}
Neurospora crassa	3.50±0.57^{a}	6.50±0.00^{b}	0.00±0.00^{b}	3.50±0.00^{ac}	6.00±0.00^{b}	6.00±0.62^{b}	3.20±0.62^{a}	5.00±0.62^{b}	5.00±0.57^{b}	6.50±0.00^{bc}	0.00±0.00^{b}
Candida albican	4.50±0.00^{ac}	5.50±0.57^{b}	5.00±0.33^{ab}	4.50±0.57^{bc}	5.50±0.62^{b}	6.50±0.62^{a}	0.20±0.00^{ab}	4.00±0.62^{ab}	5.00±0.33^{ab}	8.00±0.33^{b}	0.00±0.00^{ab}
Candida dubliniensis	4.00±0.62^{bc}	6.50±0.00^{bc}	3.50±0.57^{bc}	4.00±0.57^{ac}	6.00±0.33^{ab}	8.50±0.00^{b}	0.00±0.00^{ab}	0.50±0.00^{ac}	3.50±0.57^{bc}	8.50±0.00^{ab}	0.00±0.00^{a}
Saccharomyces rouxii	0.20±0.00^{ab}	6.50±0.00^{a}	4.00±0.62^{ab}	5.50±0.00^{ab}	6.00±0.33^{a}	7.50±0.33^{a}	4.50±0.62^{b}	0.30±0.00^{ab}	4.50±0.25^{b}	6.50±0.33^{bc}	0.00±0.00^{a}

Table 6: Antimicrobial Sensitivity Pattern of the Tested Microorganisms against Extracts.
(Zone Of Inhibition Measured In Millimeter 'Mm')
Seed Extracts of J. Curcas in Different Solvents
Data Are Presented As Mean±S.E. Value with the Same Superscript along the Column Are Not Significantly Different (P≤0.05)

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Bacteria)											
Micrococcus luteus	5.50±0.00^{b}	8.50±0.33^{bc}	4.50±0.62^{b}	5.00±0.62^{b}	7.50±0.62^{bc}	6.50±0.25^{b}	5.59±0.62^{bc}	4.50±0.33^{a}	6.00±0.00^{a}	9.50±0.00^{b}	0.00±0.00^{a}
Streptococcus pyogenes	5.00±0.62^{b}	7.00±0.00^{b}	2.00±0.57^{b}	6.00±0.00^{b}	8.50±0.00^{ac}	9.50±0.62^{b}	5.00±0.57^{ac}	5.00±0.00^{b}	5.50±0.00^{ac}	4.50±0.57^{b}	0.00±0.00^{ab}
S. aureus	2.50±0.25^{b}	9.00±0.25^{b}	3.50±0.33^{a}	5.50±0.57^{bc}	10.00±0.57^{a}	7.00±0.00^{bc}	4.50±0.57^{bc}	5.00±0.00^{b}	5.50±0.62^{bc}	10.00±0.00^{ab}	0.00±0.00^{a}
S. epidermidis	5.00±0.33^{b}	6.50±0.62^{ab}	2.00±0.00^{b}	4.50±0.57^{b}	6.50±0.57^{a}	5.00±0.33^{bc}	3.20±0.62^{a}	5.00±0.25^{b}	5.00±0.00^{ab}	11.00±0.00^{a}	0.00±0.00^{ab}
Enterobacter cloacae	4.50±0.57^{bc}	7.00±0.00^{ac}	5.50±0.57^{a}	5.00±0.33^{ab}	8.00±0.57^{a}	3.50±0.33^{a}	3.50±0.57^{a}	5.50±0.33^{bc}	6.00±0.33^{b}	7.00±0.57^{b}	0.00±0.00^{b}
P. aeruginosa	4.50±0.62^{b}	7.50±0.57^{bc}	3.50±0.62^{bc}	6.00±0.00^{b}	9.50±0.00^{ab}	5.00±0.62^{b}	4.50±0.00^{ac}	4.50±0.62^{b}	5.00±0.62^{b}	9.50±0.57^{bc}	0.00±0.00^{a}
Salmonella tphyil	5.00±0.57^{b}	6.00±0.57^{ac}	5.50±0.57^{ac}	6.00±0.25^{b}	7.50±0.57^{ac}	5.00±0.57^{b}	4.00±0.62^{bc}	3.50±0.00^{b}	6.00±0.00^{b}	8.00±0.33^{bc}	0.00±0.00^{bc}
Escherichia coli	6.50±0.33^{a}	6.50±0.57^{a}	4.50±0.62^{a}	5.00±0.62^{bc}	6.00±0.33^{b}	8.00±0.57^{ac}	0.20±0.00^{ab}	5.00±0.62^{ab}	5.00±0.33^{bc}	10.50±0.62^{b}	0.00±0.00^{b}
Shigella flexineri	5.00±0.00^{b}	7.50±0.00^{ab}	4.59±0.57^{b}	4.50±0.57^{bc}	6.00±0.62^{b}	5.50±0.25^{b}	4.00±0.57^{bc}	4.50±0.00^{ac}	6.50±0.57^{b}	11.00±0.33^{bc}	0.00±0.00^{ab}
Klebsiella pneumonia	4.50±0.33^{bc}	8.00±0.25^{b}	2.50±0.62^{b}	6.00±0.00^{a}	6.00±0.00^{b}	6.00±0.62^{b}	0.00±0.00^{ac}	4.50±0.57^{ac}	5.50±0.57^{bc}	6.50±0.57^{bc}	0.00±0.00^{a}
Pseudomonas putidae	6.00±0.00^{b}	9.50±0.33^{b}	4.50±0.57^{b}	5.00±0.62^{bc}	7.00±0.62^{b}	6.00±0.62^{b}	4.50±0.33^{a}	5.00±0.62^{a}	6.00±0.00^{b}	5.50±0.25^{b}	0.00±0.00^{a}

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Fungi)											
S. cerevisiae	6.00±0.33^{bc}	8.50±0.00^{bc}	4.20±0.62^{bc}	6.67±0.57^{ac}	6.00±0.00^{bc}	6.50±0.57^{bc}	4.50±0.33^{a}	5.50±0.57^{ac}	6.00±0.57^{a}	6.00±0.62^{b}	0.00±0.00^{bc}
Neurospora crassa	5.00±0.62^{b}	8.00±0.00^{b}	3.00±0.00^{b}	6.00±0.62^{bc}	6.50±0.25^{b}	5.00±0.57^{ac}	0.00±0.00^{ab}	6.00±0.00^{ab}	5.00±0.57^{ac}	4.50±0.62^{a}	0.00±0.00^{b}
Candida albican	5.50±0.00^{b}	6.50±0.00^{b}	5.00±0.00^{b}	5.59±0.57^{ac}	6.50±0.57^{bc}	4.50±0.62^{a}	5.00±0.57^{ac}	5.00±0.00^{a}	5.50±0.00^{ab}	6.50±0.57^{bc}	0.00±0.00^{ab}
Candida dubliniensis	5.00±0.57^{b}	6.50±0.57^{b}	4.50±0.33^{bc}	6.00±0.62^{ab}	7.00±0.33^{ab}	4.50±0.62^{b}	3.50±0.00^{ac}	3.50±0.57^{a}	6.00±0.25^{b}	5.00±0.57^{ac}	0.00±0.00^{a}
Saccharomyces rouxii	4.50±0.33^{bc}	6.00±0.62^{b}	3.00±0.00^{b}	7.00±0.00^{ab}	6.00±0.00^{bc}	5.00±0.57^{b}	5.59±0.62^{bc}	3.00±0.00^{a}	6.50±0.00^{b}	6.00±0.62^{b}	0.00±0.00^{a}

Table 7: Antimicrobial Sensitivity Pattern of the Tested Microorganisms Againstextracts.
(Zone Of Inhibition Measured In Millimeter 'Mm')
Seed Extracts of N. Tabacum In Different Solvents
Data Are Presented As Mean±S.E. Value with the Same Superscript along the Column Are Not Significantly Different (P≤0.05)

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Bacteria)											
Micrococcus luteus	7.50±0.33^{b}	12.00±0.62^{b}	4.50±0.62^{a}	7.50±0.57^{a}	13.00±0.33^{b}	8.50±0.57^{b}	3.50±0.62^{bc}	4.50±0.62^{b}	6.00±0.62^{b}	7.59±0.57^{b}	0.00±0.00^{a}
Streptococcus pyogenes	8.50±0.00^{bc}	11.52±0.57^{b}	4.59±0.57^{b}	7.00±0.00^{ac}	11.50±0.62^{b}	10.00±0.00^{b}	5.00±0.57^{a}	6.00±0.33^{a}	5.00±0.33^{bc}	9.67±0.00^{b}	0.00±0.00^{a}
S. aureus	7.50±0.62^{b}	8.50±0.00^{b}	2.50±0.62^{b}	5.58±0.62^{a}	8.50±0.33^{a}	7.59±0.57^{b}	3.00±0.33^{bc}	5.00±0.33^{a}	6.00±0.00^{b}	9.50±0.00^{b}	0.00±0.00^{ac}

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
S. epidermidis	5.50±0.57^{b}	12.00±0.25^{b}	4.50±0.57^{b}	6.50±0.57^{a}	10.20±0.33^{ab}	9.67±0.00^{b}	5.00±0.00^{ab}	4.50±0.33^{b}	5.50±0.33^{a}	8.00±0.33^{a}	0.00±0.00^{ac}
Enterobacter cloacae	7.00±0.33^{ab}	10.00±0.62^{a}	3.00±0.00^{b}	5.50±0.00^{bc}	9.50±0.33^{a}	9.50±0.00^{b}	4.50±0.62^{a}	6.00±0.33^{ab}	4.50±0.00^{b}	8.00±0.62^{b}	0.00±0.00^{ab}
P. aeruginosa	6.00±0.33^{a}	9.56±0.57^{a}	5.20±0.62^{bc}	4.50±0.25^{b}	12.00±0.33^{a}	8.00±0.33^{a}	6.00±0.00^{ac}	4.50±0.00^{b}	5.20±0.33^{bc}	7.50±0.57^{bc}	0.00±0.00^{a}
Salmonella tphyil	7.00±0.57^{b}	10.00±0.00^{ab}	5.00±0.57^{a}	6.50±0.57^{b}	10.50±0.00^{b}	8.00±0.62^{b}	4.59±0.57^{b}	6.50±0.00^{b}	6.00±0.62^{b}	9.50±0.62^{ab}	0.00±0.00^{ab}
Escherichia coli	6.50±0.33^{a}	11.50±0.00^{ac}	4.50±0.62^{b}	6.00±0.00^{b}	8.50±0.00^{b}	7.50±0.57^{bc}	3.50±0.62^{bc}	5.50±0.33^{b}	5.50±0.00^{bc}	10.00±0.00^{ab}	0.00±0.00^{a}
Shigella flexineri	7.52±0.00^{b}	12.00±0.62^{ab}	3.00±0.33^{bc}	7.50±0.33^{ab}	11.00±0.33^{b}	9.50±0.62^{ab}	2.50±0.62^{b}	6.00±0.00^{ac}	4.20±0.00^{a}	11.0±0.00^{ac}	0.00±0.00^{ab}
Klebsiella pneumonia	6.53±0.33^{bc}	8.23±0.00^{ab}	3.50±0.62^{bc}	6.50±0.00^{b}	9.00±0.62^{b}	10.00±0.00^{ab}	3.50±0.62^{bc}	5.00±0.62^{a}	6.50±0.57^{a}	6.00±0.00^{a}	0.00±0.00^{b}
Pseudomonas putidae	7.50±0.00^{b}	10.54±0.57^{a}	2.00±0.33^{bc}	6.00±0.33^{b}	9.50±0.00^{ac}	5.20±0.33^{bc}	4.50±0.62^{a}	5.50±0.00^{a}	3.20±0.62^{ab}	7.00±0.62^{bc}	0.00±0.00^{ab}
Test Organisms (Fungi)											
S. cerevisiae	4.50±0.00^{bc}	5.24±0.00^{ac}	4.50±0.62^{a}	2.50±0.57^{ac}	6.00±0.00^{a}	5.50±0.00^{bc}	2.50±0.25^{b}	2.50±0.00^{a}	3.00±0.00^{ab}	4.50±0.00^{ac}	0.00±0.00^{ab}

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Neurospora crassa	5.00 ± 0.33^{b}	5.25 ± 0.57^{ac}	4.59 ± 0.57^{b}	4.00 ± 0.62^{ab}	4.50 ± 0.57^{bc}	5.20 ± 0.62^{bc}	5.00 ± 0.33^{b}	4.00 ± 0.57^{ac}	2.50 ± 0.00^{bc}	5.00 ± 0.57^{ac}	0.00 ± 0.00^{a}
Candida albican	4.50 ± 0.62^{b}	6.00 ± 0.62^{a}	2.50 ± 0.62^{b}	4.50 ± 0.00^{a}	5.50 ± 0.25^{b}	5.00 ± 0.57^{a}	4.50 ± 0.57^{bc}	2.50 ± 0.00^{bc}	1.50 ± 0.33^{a}	3.50 ± 0.62^{bc}	0.00 ± 0.00^{ab}
Candida dubliniensis	7.50 ± 0.00^{b}	7.00 ± 0.00^{a}	4.50 ± 0.57^{b}	3.50 ± 0.57^{ac}	5.00 ± 0.00^{b}	4.50 ± 0.62^{b}	4.50 ± 0.62^{b}	3.50 ± 0.62^{ab}	4.00 ± 0.33^{bc}	4.50 ± 0.00^{a}	0.00 ± 0.00^{a}
Saccharomyces rouxii	5.00 ± 0.33^{b}	5.54 ± 0.57^{bc}	4.59 ± 0.57^{b}	4.50 ± 0.00^{ac}	1.50 ± 0.62^{b}	3.00 ± 0.33^{bc}	2.50 ± 0.25^{b}	3.00 ± 0.33^{b}	2.00 ± 0.33^{a}	3.50 ± 0.57^{ac}	0.00 ± 0.00^{ab}

Table 8: Antimicrobial sensitivity pattern of the tested microorganisms against extracts (Zone of inhibition measured in millimeter 'mm') Leafextracts of J. curcas in different solvents

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Bacteria)											
Micrococcus luteus	5.20 ± 0.33^{ab}	7.50 ± 0.62^{b}	6.00 ± 0.00^{b}	6.50 ± 0.62^{b}	11.00 ± 0.62^{a}	10.00 ± 0.00^{ab}	5.50 ± 0.33^{bc}	4.50 ± 0.33^{ab}	6.00 ± 0.33^{bc}	7.50 ± 0.62^{bc}	0.00 ± 0.00^{ab}
Streptococcus pyogenes	5.00 ± 0.25^{b}	8.00 ± 0.00^{b}	5.50 ± 0.57^{bc}	6.00 ± 0.00^{b}	8.50 ± 0.57^{a}	11.00 ± 0.00^{a}	6.00 ± 0.57^{bc}	6.00 ± 0.33^{b}	5.50 ± 0.62^{b}	8.50 ± 0.00^{ac}	0.00 ± 0.00^{a}
S. aureus	5.00 ± 0.33^{ab}	6.50 ± 0.33^{b}	4.50 ± 0.57^{b}	5.00 ± 0.33^{a}	8.00 ± 0.00^{ab}	7.00 ± 0.57^{b}	7.50 ± 0.00^{b}	5.00 ± 0.33^{a}	6.00 ± 0.33^{bc}	10.00 ± 0.57^{a}	0.00 ± 0.00^{a}
S. epidermidis	4.50 ± 0.33^{a}	9.20 ± 0.33^{bc}	5.00 ± 0.33^{ab}	6.00 ± 0.33^{b}	9.50 ± 0.00^{ac}	9.50 ± 0.57^{bc}	6.50 ± 0.25^{b}	4.50 ± 0.25^{b}	6.65 ± 0.00^{b}	6.50 ± 0.57^{a}	0.00 ± 0.00^{ac}
Enterobacter cloacae	6.50 ± 0.62^{b}	6.50 ± 0.25^{b}	6.00 ± 0.00^{b}	4.00 ± 0.33^{ab}	9.00 ± 0.62^{a}	8.00 ± 0.33^{bc}	5.00 ± 0.62^{b}	6.00 ± 0.00^{b}	5.00 ± 0.62^{b}	8.00 ± 0.57^{a}	0.00 ± 0.00^{ac}

Saccharomyces rouxii	*Candida dubliniensis*	*Candida albican*	*Neurospora crassa*	*S. cerevisiae*	**Test Organisms (Fungi)**	*Pseudomonas putidae*	*Klebsiella pneumonia*	*Shigella flexineri*	*Escherichia coli*	*Salmonella tphyil*	*P. aeruginosa*
5.00±0.57^{b}	6.00±0.33^{b}	7.50±0.33^{a} $_{b}$	6.50±0.62^{b}	6.00±0.00^{a} $_{b}$		6.00±0.62^{bc}	4.00±0.00^{ac}	6.00±0.00^{a}	4.00±0.62^{b}	5.50±0.00^{bc}	4.50±0.25^{b}
5.00±0.33^{b}	7.50±0.00^{ac}	7.00±0.57^{b} $_{c}$	7.00±0.00^{bc}	8.50±0.00^{a} $_{c}$		8.50±0.33^{a}	6.25±0.57^{b}	9.50±0.00^{b}	8.00±0.33^{a}	7.75±0.25^{b}	7.50±0.57^{b}
6.00±0.00^{a}	4.00±0.62^{b}	5.50±0.00^{b} $_{c}$	4.50±0.25^{b}	6.00±0.62^{b} $_{c}$		6.67±0.57^{ac}	5.00±0.62^{bc}	6.00±0.00^{a}	4.50±0.57^{bc}	5.00±0.62^{bc}	6.00±0.25^{b}
6.00±0.33^{b}	6.00±0.33^{ab}	7.52±0.33^{a}	7.00±0.33^{b}	5.00±0.00^{b} $_{c}$		5.00±0.00^{ac}	5.00±0.62^{bc}	6.50±0.62^{b}	6.00±0.62^{b}	6.00±0.33^{ab}	4.50±0.00^{b}
5.00±0.25^{b}	6.00±0.00^{bc}	6.50±0.33^{b}	6.00±0.25^{b}	6.50±0.00^{b}		8.50±0.00^{b}	8.00±0.33^{b}	10.00±0.33^{ab}	8.50±0.00^{b}	9.50±0.33^{a}	10.50±0.00^{ab}
4.50±0.57^{bc}	5.00±0.62^{bc}	6.00±0.25^{b}	6.00±0.00^{b}	5.00±0.33^{a} $_{b}$		7.50±0.57^{ac}	6.50±0.62^{a}	9.50±0.57^{bc}	6.50±0.57^{bc}	11.00±0.33^{bc}	10.50±0.62^{b}
5.00±0.33^{ab}	4.00±0.62^{b}	4.50±0.62^{b}	5.00±0.33^{b}	4.50±0.00^{b} $_{c}$		6.00±0.33^{b}	4.50±0.62^{a}	6.50±0.25^{b}	8.50±0.00^{ab}	6.00±0.62^{b}	6.50±0.57^{bc}
4.50±0.33^{a}	6.50±0.62^{b}	5.00±0.33^{b}	6.50±0.62^{b}	6.00±0.00^{b} $_{c}$		5.50±0.33^{bc}	4.50±0.33^{b}	6.00±0.25^{b}	5.00±0.33^{bc}	5.50±0.33^{b}	4.50±0.00^{b}
2.50±0.33^{a}	4.00±0.00^{ab}	3.50±0.00^{a} $_{b}$	3.50±0.57^{ac}	5.50±0.00^{a} $_{b}$		4.00±0.57^{ac}	6.65±0.00^{ab}	5.50±0.00^{ac}	5.00±0.62^{bc}	6.65±0.00^{b}	5.20±0.33^{ab}
4.50±0.00^{b}	6.00±0.00^{b}	4.50±0.25^{b}	5.00±0.33^{a}	6.00±0.00^{b} $_{c}$		7.00±0.62^{b}	6.00±0.00^{b}	6.00±0.62^{b}	6.00±0.33^{b}	7.50±0.57^{ac}	9.50±0.00^{ab}
0.00±0.00^{a}	0.00±0.00^{ab}	0.00±0.00^{a} $_{b}$	0.00±0.00^{b}	0.00±0.00^{b} $_{c}$		0.00±0.00^{b}	0.00±0.00^{ab}	0.00±0.00^{a}	0.00±0.00^{ab}	0.00±0.00^{a}	0.00±0.00^{ab}

Table 9: Antimicrobial Sensitivity Pattern of the Tested Microorganisms Againstextracts.
(Zone Of Inhibition Measured In Millimeter 'Mm')
Leaf Extracts of N. Tabacum in Differentsolvents

Data Are Presented as Mean±S.E. Value With The Same Superscript Along the Column Are Not Significantly Different (P≤0.05).

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Bacteria)											
Micrococcus luteus	4.50±0.33^{bc}	6.50±0.00^{b}	2.50±0.62^{b}	5.00±0.62^{b}	11.50±0.62^{bc}	7.50±0.00^{b}	2.00±0.57^{b}	5.50±0.33^{a}	6.50±0.62^{b}	6.00±0.62^{b}	0.00±0.00^{ac}
Streptococcus pyogenes	5.50±0.33^{ab}	7.00±0.25^{b}	4.50±0.57^{b}	6.00±0.00^{b}	8.00±0.57^{ac}	6.50±0.25^{b}	3.50±0.33^{a}	5.00±0.33^{ab}	4.50±0.33^{bc}	5.00±0.33^{bc}	0.00±0.00^{ac}
S. aureus	4.50±0.00^{b}	6.65±0.33^{b}	3.00±0.00^{b}	5.50±0.33^{bc}	9.00±0.57^{ac}	5.00±0.62^{b}	2.00±0.00^{b}	6.50±0.33^{bc}	6.00±0.33^{a}	6.00±0.00^{b}	0.00±0.00^{ab}
S. epidermidis	5.00±0.33^{a}	7.50±0.33^{ab}	5.20±0.62^{bc}	5.00±0.62^{b}	7.50±0.00^{ab}	6.50±0.57^{bc}	5.50±0.57^{a}	6.00±0.00^{b}	5.00±0.62^{b}	5.50±0.33^{a}	0.00±0.00^{a}
Enterobacter cloacae	6.00±0.33^{a}	6.50±0.00^{bc}	5.00±0.57^{a}	6.50±0.33^{a}	8.00±0.00^{a}	6.00±0.62^{b}	3.50±0.62^{bc}	6.50±0.62^{b}	5.50±0.00^{b}	4.50±0.00^{b}	0.00±0.00^{ab}
P. aeruginosa	3.50±0.25^{b}	6.00±0.00^{b}	4.50±0.62^{b}	4.20±0.00^{ac}	9.50±0.62^{bc}	8.50±0.00^{ab}	5.50±0.57^{ac}	6.50±0.62^{b}	4.00±0.62^{b}	5.20±0.33^{bc}	0.00±0.00^{a}
Salmonella tphyil	4.58±0.57^{b}	6.50±0.57^{b}	3.00±0.33^{bc}	5.50±0.00^{ac}	11.00±0.00^{ac}	6.50±0.25^{b}	4.50±0.62^{a}	6.50±0.62^{b}	5.50±0.33^{bc}	6.00±0.62^{b}	0.00±0.00^{ab}
Escherichia coli	3.50±0.62^{b}	5.50±0.00^{bc}	3.50±0.62^{bc}	6.00±0.57^{bc}	8.50±0.33^{ab}	4.50±0.62^{a}	4.59±0.57^{b}	6.50±0.62^{b}	5.50±0.00^{b}	5.50±0.00^{bc}	0.00±0.00^{b}
Shigella flexineri	5.50±0.33^{bc}	7.00±0.33^{ab}	2.00±0.33^{bc}	6.50±0.00^{ab}	7.50±0.00^{b}	6.00±0.33^{b}	2.50±0.62^{b}	6.50±0.62^{b}	6.00±0.62^{bc}	6.50±0.57^{bc}	0.00±0.00^{ab}

Saccharomyces rouxii	*Candida dubliniensis*	*Candida albican*	*Neurospora crassa*	*S. cerevisiae*	Test Organisms (Fungi)	*Pseudomonas putidae*	*Klebsiella pneumonia*
4.50±0.00^{bc}	5.00±0.33^{bc}	4.50±0.00^{bc}	5.20±0.00^{ac}	4.00±0.62^{a}		4.50±0.33^{bc}	5.00±0.00^{b}
5.50±0.33^{b}	4.50±0.00^{ab}	5.50±0.57^{a}	5.00±0.57^{bc}	4.50±0.00^{ac}		6.50±0.33^{a}	8.00±0.33^{a}
4.00±0.57^{a}	2.50±0.57^{ac}	3.00±0.33^{bc}	4.50±0.57^{b}	2.50±0.62^{b}		4.50±0.62^{a}	4.59±0.57^{b}
3.50±0.33^{bc}	4.00±0.33^{a}	5.90±0.25^{b}	5.50±0.00^{b}	4.50±0.62^{b}		6.00±0.57^{b}	5.00±0.62^{ab}
6.50±0.25^{b}	5.50±0.00^{ac}	6.20±0.57^{bc}	7.50±0.00^{bc}	8.00±0.00^{a}		11.50±0.00^{b}	8.50±0.33^{b}
5.00±0.57^{a}	5.20±0.62^{bc}	3.00±0.00^{b}	4.50±0.57^{b}	5.00±0.33^{b}		4.50±0.00^{bc}	6.00±0.57^{bc}
4.20±0.62^{bc}	3.00±0.00^{b}	4.50±0.33^{bc}	5.00±0.00^{b}	3.00±0.00^{b}		5.00±0.33^{b}	4.50±0.57^{b} \
3.50±0.00^{bc}	4.00±0.57^{bc}	5.00±0.00^{b}	4.50±0.62^{b}	5.00±0.33^{a}		5.50±0.57^{b}	6.50±0.62^{b}
4.50±0.33^{ab}	5.00±0.25^{b}	4.00±0.57^{a}	2.50±0.57^{ac}	4.50±0.00^{a}		5.50±0.00^{ac}	4.50±0.62^{bc}
4.50±0.33^{bc}	5.00±0.00^{b}	5.50±0.33^{bc}	3.50±0.62^{b}	4.58±0.57^{b}		8.50±0.00^{ab}	6.00±0.62^{b}
0.00±0.00^{ab}	0.00±0.00^{a}	0.00±0.00^{ab}	0.00±0.00^{a}	0.00±0.00^{ab}		0.00±0.00^{ab}	0.00±0.00^{a}

Table 10: Antimicrobial Sensitivity Pattern of the Tested Microorganisms against Extracts.
(Zone Of Inhibition Measured In Millimeter 'Mm')
Stem Extracts of J. Curcas in Different Solvents
Data Are Presented As Mean±S.E. Value with the Same Superscript along the Column Are Not Significantly Different (P≤0.05).

	Water		% of Ethanolic solvent				% n-Hexane solvent				Control
Isolates	**Hot**	**Ordinary**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**20%Tween 20**
Test Organisms (Bacteria)											
Micrococcus luteus	3.50 ± 0.33^{bc}	6.00 ± 0.62^{bc}	2.50 ± 0.62^{b}	4.50 ± 0.00^{a}	8.50 ± 0.57^{a}	6.00 ± 0.57^{bc}	4.50 ± 0.00^{bc}	4.00 ± 0.33^{a}	5.00 ± 0.57^{b}	5.00 ± 0.33^{a}	0.00 ± 0.00^{a}
Streptococcus pyogenes	5.00 ± 0.33^{bc}	6.00 ± 0.00^{ac}	4.50 ± 0.57^{b}	5.00 ± 0.00^{bc}	8.00 ± 0.00^{ab}	7.50 ± 0.00^{b}	3.50 ± 0.33^{a}	4.50 ± 0.00^{b}	5.00 ± 0.57^{b}	6.00 ± 0.33^{b}	0.00 ± 0.00^{ac}
S. aureus	4.00 ± 0.33^{b}	5.50 ± 0.00^{ac}	3.00 ± 0.00^{b}	6.00 ± 0.00^{b}	9.00 ± 0.00^{ac}	6.50 ± 0.25^{b}	2.00 ± 0.00^{b}	5.50 ± 0.33^{bc}	4.59 ± 0.57^{ac}	4.00 ± 0.33^{ab}	0.00 ± 0.00^{ac}
S. epidermidis	4.00 ± 0.57^{b}	6.20 ± 0.57^{bc}	5.20 ± 0.62^{bc}	5.00 ± 0.57^{ac}	7.20 ± 0.33^{a}	5.00 ± 0.62^{b}	5.50 ± 0.57^{a}	4.50 ± 0.62^{b}	5.00 ± 0.00^{ax}	4.50 ± 0.00^{b}	0.00 ± 0.00^{ab}
Enterobacter cloacae	5.20 ± 0.62^{b}	4.50 ± 0.62^{b}	5.00 ± 0.57^{a}	6.50 ± 0.57^{ac}	7.50 ± 0.00^{b}	6.50 ± 0.57^{bc}	3.50 ± 0.62^{bc}	5.50 ± 0.00^{b}	4.00 ± 0.00^{ab}	6.00 ± 0.33^{ab}	0.00 ± 0.00^{a}
P. aeruginosa	3.00 ± 0.57^{a}	4.50 ± 0.33^{bc}	4.50 ± 0.62^{b}	5.50 ± 0.00^{ab}	8.00 ± 0.33^{bc}	6.00 ± 0.62^{b}	5.50 ± 0.57^{ac}	5.00 ± 0.57^{b}	3.50 ± 0.57^{ac}	6.00 ± 0.62^{b}	0.00 ± 0.00^{ab}
Salmonella tphyil	4.00 ± 0.57^{ac}	5.50 ± 0.00^{b}	3.00 ± 0.33^{bc}	5.50 ± 0.00^{ac}	9.50 ± 0.33^{bc}	8.50 ± 0.00^{ab}	4.50 ± 0.62^{a}	6.00 ± 0.33^{ab}	4.50 ± 0.00^{ab}	6.50 ± 0.62^{b}	0.00 ± 0.00^{a}
Escherichia coli	4.00 ± 0.33^{b}	4.00 ± 0.62^{a}	3.50 ± 0.62^{bc}	6.00 ± 0.62^{b}	7.50 ± 0.33^{b}	6.50 ± 0.25^{b}	4.59 ± 0.57^{b}	4.00 ± 0.00^{b}	4.50 ± 0.57^{ac}	5.00 ± 0.62^{bc}	0.00 ± 0.00^{ab}
Shigella flexineri	5.20 ± 0.62^{b}	6.20 ± 0.57^{ac}	2.00 ± 0.33^{bc}	6.50 ± 0.00^{b}	7.00 ± 0.62^{b}	4.50 ± 0.62^{a}	2.50 ± 0.62^{b}	4.20 ± 0.25^{b}	5.00 ± 0.33^{ab}	5.00 ± 0.00^{ac}	0.00 ± 0.00^{b}

Klebsiella pneumonia	4.50±0.00^{b}	5.00±0.00^{ab}	4.59±0.57^{b}	5.50±0.33^{b}	6.50±0.33^{bc}	6.00±0.33^{b}	4.50±0.57^{b}\	5.00±0.00^{b}	4.50±0.33^{b}	7.00±0.33^{b}	0.00±0.00^{ab}
Pseudomonas putidae	4.50±0.62^{b}	5.00±0.57^{bc}	4.50±0.62^{a}	5.50±0.00^{bc}	7.80±0.33^{ab}	6.00±0.00^{b}	4.20±0.62^{bc}	3.50±0.33^{bc}	4.50±0.33^{bc}	5.00±0.00^{bc}	0.00±0.00^{ab}
Test Organisms (Fungi)											
S. cerevisiae	6.50±0.00^{b}	5.60±0.00^{ab}	2.50±0.62^{b}	5.50±0.62^{b}	8.00±0.33^{bc}	5.00±0.33^{b}	3.00±0.00^{b}	6.00±0.57^{b}	5.50±0.25^{b}	7.52±0.33^{a}	0.00±0.00^{a}
Neurospora crassa	6.00±0.57^{b}	6.00±0.57^{ac}	4.50±0.57^{b}	6.00±0.00^{b}	7.50±0.00^{b}	4.50±0.62^{b}	5.00±0.00^{b}	5.90±0.00^{bc}	5.00±0.57^{bc}	6.00±0.33^{ab}	0.00±0.00^{ab}
Candida albican	5.50±0.33^{a}	6.90±0.33^{a}	4.59±0.57^{b}	5.90±0.57^{b}	6.20±0.57^{b}	4.00±0.62^{b}	4.50±0.33^{bc}	6.50±0.33^{b}	6.00±0.00^{b}	6.00±0.33^{b}	0.00±0.00^{a}
Candida dubliniensis	6.56±0.33^{bc}	6.50±0.33^{bc}	4.50±0.00^{b}	5.50±0.62^{b}	6.00±0.33^{bc}	5.00±0.33^{ab}	3.00±0.00^{b}	5.50±0.62^{b}	6.50±0.00^{b}	5.00±0.62^{b}	0.00±0.00^{ab}
Saccharomyces rouxii	5.50±0.00^{b}	5.50±0.62^{ab}	4.50±0.62^{b}	5.60±0.57^{a}	6.50±0.00^{bc}	4.59±0.57^{b}	0.00±0.00^{ab}	5.00±0.57^{b}	5.69±0.00^{bc}	2.50±0.62^{b}	0.00±0.00^{ab}

Table 11: Antimicrobial Sensitivity Pattern of the Tested Microorganisms against Extracts.
(Zone of Inhibition Measured In Millimeter 'Mm')
Stem Extracts of N. Tabacum in Differentsolvents
Data Are Presented As Mean±S.E. Value with the Same Superscript along the Column Are Not Significantly Different

Standard Antibiotics	Micrococcus luteus	Streptococcus pyogenes	Staphylococcus aureus	Enterobacter cloacae	Staphylococcus epidermidis	Shigella flexineri	Klebsiella pneumonia	Pseudomonas putidae	Escherichia coli	Salmonella typhi	Pseudomonas aeruginosa
Augmentin(30µg)	8.54±0.33^{bc}	7.50±0.62^{a}	8.69±0.25^{b}	11.00±0.00^{a}	12.50±0.00^{bc}	6.50±0.25^{b}	7.50±0.33^{a}	8.00±0.25^{b}	9.00±0.57^{ac}	8.60±0.00^{ac}	7.50±0.00^{bc}
Pefloxacin(30 µg)	7.00±0.62^{b}	5.65±0.62^{bc}	6.00±0.62^{b}	7.85±0.00^{a}	6.53±0.00^{b}	8.50±0.00^{b}	8.00±0.33^{ab}	6.50±0.00^{b}	8.20±0.00^{ab}	6.50±0.00^{a}	7.20±0.57^{b}
Erithromycin (10µg)	9.50±0.33^{b}	9.65±0.57^{a}	8.00±0.33^{a}	10.56±0.57^{bc}	9.56±0.33^{ab}	9.56±0.33^{a}	10.50±0.33^{b}	13.00±0.33^{ab}	11.20±0.57^{ac}	10.50±0.00^{ac}	11.20±0.00^{b}
Streptomycin(30µg)	6.50±0.00^{b}	10.65±0.00^{ac}	7.00±0.00^{b}	95.60±0.00^{ab}	8.00±0.00^{b}	11.00±0.33^{b}	12.50±0.00^{bc}	9.50±0.33^{b}	8.35±0.57^{bc}	8.56±0.62^{ab}	8.50±0.33^{a}
Ciproflaxin (10µg)	7.80±0.33^{bc}	8.00±0.57^{ac}	6.50±0.33^{bc}	6.50±0.00^{ab}	6.50±0.33^{ab}	5.56±0.33^{ab}	6.50±0.33^{ab}	6.50±0.33^{ab}	6.57±0.00^{a}	6.00±0.57^{ac}	6.50±0.62^{b}
Zinnacef(20 µg)	6.00±0.33^{a}	6.50±0.00^{a}	6.00±0.33^{ab}	9.00±0.57^{ac}	7.50±0.33^{b}	8.00±0.33^{bc}	6.00±0.62^{b}	7.50±0.33^{bc}	6.00±0.62^{ab}	7.00±0.00^{ab}	8.00±0.00^{b}
Gentamycin(10µg)	8.90±0.33^{b}	9.50±0.57^{bc}	9.78±0.57^{b}	11.20±0.62^{b}	11.50±0.62^{b}	9.56±0.33^{b}	8.59±0.33^{b}	8.00±0.00^{b}	9.58±0.00^{ac}	12.25±0.57^{ac}	12.50±0.33^{b}
Tarivid(10 µg)	9.32±0.00^{a}	6.00±0.33^{bc}	7.53±0.57^{bc}	7.00±0.33^{bc}	5.69±0.00^{ab}	6.00±0.00^{b}	6.50±0.33^{bc}	6.00±0.33^{bc}	8.20±0.57^{bc}	5.86±0.62^{a}	6.50±0.62^{b}
Septrin(30 µg)	8.00±0.57^{bc}	8.50±0.00^{b}	6.00±0.57^{b}	6.50±0.33^{bc}	7.86±0.00^{a}	7.50±0.33^{ab}	7.00±0.33^{a}	7.50±0.33^{a}	7.50±0.00^{ab}	7.50±0.00^{ab}	8.00±0.33^{a}
Ampiclox (30µg)	9.50±0.62^{bc}	9.00±0.33^{ab}	6.00±0.33^{ab}	9.56±0.33^{b}	9.56±0.00^{ac}	7.00±0.33^{bc}	8.50±0.33^{b}	6.20±0.00^{bc}	9.56±0.57^{a}	9.85±0.33^{a}	6.50±0.33^{ab}
Amoxacillin (30µg)	6.95±0.00^{a}	7.50±0.33^{b}	8.50±0.00^{ab}	9.80±0.00^{b}	10.00±0.33^{ab}	8.20±0.33^{a}	7.56±0.33^{bc}	6.59±0.33^{bc}	9.56±0.57^{a}	8.50±0.33^{ab}	7.50±0.33^{bc}
Roceptin(25µg)	7.50±0.00^{ac}	6.50±0.33^{bc}	6.89±0.62^{bc}	8.20±0.33^{a}	7.20±0.33^{ab}	6.50±0.33^{ab}	6.50±0.33^{bc}	6.00±0.62^{b}	6.54±0.57^{a}	8.50±0.33^{ab}	7.50±0.00^{ac}

Table 12: Antimicrobial Sensitivity Pattern of the Tested Microorganisms against Standard Antibiotics. (Zone Of Inhibition Measured In Millimeter 'Mm')

Test Organisms (Bacteria) Data Are Presented as Mean±S.E. Value with the Same Superscript along the Column Are Not Significantly Different (P≤0.05)

Standard Antibiotics	Saccharomyces Cerevisiae	Neurospora Crassa	Candida Albicans	Candida Dubliniensis	Saacharomyces Rouxii
Ketoconazole(20mg)	13.5±0.33^{b}	11.20±0.33^{ab}	10.59±0.00^{bc}	12.59±0.00^{b}	12.50±0.00^{b}
Fluconazole(20mg)	11.52±0.33^{ab}	12.00±0.00^{b}	11.50±0.33^{bc}	11.00±0.00^{bc}	12.00±0.57^{bc}
Voriconazole(20 µg)	12.50±0.33^{b}	10.59±0.00^{ab}	13.00±0.00^{bc}	12.59±0.33^{bc}	10.58±0.62^{b}

Table 13: Antimicrobial Sensitivity Pattern of the Identified Sequenced Tested Microorganisms against Commercial Standard Antibiotics (Zone Of Inhibition Measured In Millimeter 'Mm') Test Organisms (Fungi) Data Are Presented As Mean ± S.E. Value with the Same Superscript along the Column Are Not Significantly Different (P≤0.05)

4. Discussion

Different parts of the plant extracts used were constituted at varying concentration and showed different antimicrobial activities against tested microorganisms. The effect of the solvents used for the extraction of the plant extracts were also observed in relation with the effectiveness of the extract to exhibit antimicrobial activities against tested microorganisms. Extracts exhibited varying degrees of antimicrobial efficacy against the bacterial and fungal isolates. Generally, the antimicrobial efficacy of the extracts increased with a corresponding increase in extract concentration. The bioactive potential of the extracts also varied with extraction solvents. Extract prepared with hot water was more effective than cold water and ethanolic extracts shows higher antimicrobial potential than other solvents. This revealed the effect that temperature can impact on the activity and derivation of the bioactive compound, it show the tendency of using hot water as a suitable extraction solvent in order to obtain active and participatory antimicrobial agents that inherent in the extract that can produce effective inhibition. This specifically indicate the tendency of using hot water as a suitable extraction solvent in order to obtain active and participatory antimicrobial agents inherent in the extract which led to an effective inhibition on the isolates. Ethanol rated best as the extraction solvent, followed by hot water, n- Hexane and cold water in that order. The extracts were found to induce remarkable antimicrobial potential against the test organisms, most especially the hot water and ethanolic extracts with varying ranges of inhibition against the isolates.

Ethanolic root extract of *J. curcas* at 75% and 100% extracts concentration inhibiting the growth of the tested microorganisms as shown in*Pseudomonas putidae* and *Enterobacter cloaca* as the most susceptible at 11.0±0.00mm and 11.00±0.00mm zone of inhibition respectively while the n-hexane solvent extracts of the same part of the plants at the same concentration against *Pseudomonas putidae* and *Enterobacter cloacae* with 5.50±0.25mm and 5.00±0.00mm zone of inhibition respectively (Table 5). *S.aureus* and *Micrococcus luteus* is most susceptible to ethanolic seed extracts of*Jatropha curcas* with 12.00±0.00mm and 13.00±0.33mm zone of inhibition respectively (Table 6). Extraction of the plant extracts at any part of the plants body had proven to be more effective as antimicrobial agents against tested microorganisms using ethanol best at 75% (w/v) concentration of the extracts than the use of n-hexane and hot water solvent extraction (Table 7). Ethanolic leaf extracts at 75% concentration of *Jatropha curcas* shows to be the most effective against *P. aeruginosa* with 12.00±0.33mm zone of inhibition, but least susceptible in the same concentration using n-hexane extracts with 5.20±0.33m zone of inhibition (Table 8). In a broad consideration, *Jatropha curcas* extracts had shown to be more effective in inhibiting the growth of tested microorganisms compared with *Nicotiana tabacum* as antimicrobial agents (Table 9). The shafts and the stem portion of the parts of the plant body that used to control the growth of tested microorganisms in this research has not been significantly effective as an antimicrobial agent both in ethanol and n-hexane solvents(Table 11 and 12). The use of standard antibiotics (both Gram positive, Gram negative and antifungal antibiotics) has shown to be remarkably effective against tested microorganisms and favorably compared with the plant extracts used in this study (Table 13 and 14).

The regular and extensive use of modern antibiotics will lead to development of drug resistant. Today, Methicillin-resistant *Staphylococcus aureus* have become problematic in the hospital setting, and antibiotic resistance has entered the multidrug-resistant phase [Zetola*et al.,* 2005].Generally the antimicrobial potential of the extracts increased with a corresponding increase in extract concentration. The influential role of the bioactive compounds in the *J. curcas* and*N. tabacum* extracts suggested the possible treatment for the healing of the wound of diabetic patients, the metabolites played the role of inhibiting the growth of the isolates which could be contributive positively to the possible removal of the delayed healing in the case of wounds. Invariably, despite the fact that both modern standard antibiotics disc and the *J. curcas* and*N. tabacum* extracts used shows the inhibitory effect on the tested isolates. Compared with antibiotics, some resistant strain microorganisms cannot be effectively controlled by modern antibiotics but rather can do with the *J. curcas* and*N. tabacum* extracts as proven by this present study and especially to the bacterial isolates that are of gram negative which can easily developed drug resistant [Sofowora, 2006]. Antimicrobial efficacy of the extracts of *J. curcas* and*N. tabacum* evaluated in this study had been proven to be well effective and cheap preventive therapy against the microbial effects in wounds treatment that often facing antimicrobial resistance and it could be a suitable source of new antimicrobial natural product or as a base for the development of new drugs in phytomedicine.

5. Conclusion

The research revealed that with the appropriate and adequate use of the *J. curcas* and*N. tabacum* extracts that were experimented; the microbial load on the wound of diabetic patients can be minimized and drastically reduced to fasten the healing condition of the wounds. However, if timely and effectively applied, it can prevent the complication from degenerating into amputation or early death of the diabetic patients and by the way serve as a useful replacement and alternative to modern antibiotics in achieving curative and effective control against the colonized microorganisms on the wounds which could be a vital contributing factors to the delayed healing of wounds.

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7. Ethical Approval

In conformity with the required protocol, standard written ethical approval was collected from Ministry of Health, Ondo State after the introductory letter from the Department of microbiology, Federal University of Technology Akure to seek necessary consent and permission from the Ministry. Also, respective approval and acknowledgement letter from Department of Medicine- Diabetic Unit, in each of the visited hospitals were collected and preserved by the authors.

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